

120. A purified preparation of bioactive substances obtained by the method of claim 116.

121. A purified preparation of bioactive substances obtained by the method of claim 116 wherein the plant material is Kava root.

### Remarks

The new claims contain elements from the cancelled claims and also further details that more particularly describe embodiments of the claimed invention. New matter is not added. The recitation of "mechanically disrupted by grinding, or crushing to form a powder or paste" for example, is supported by the specification on p. 16 line 11 and p. 19 line 12. The recitation of "isopropyl amine as a secondary modifier" in claim 99 is supported, for example, by the specification on page 38 lines 23-26. The recitation of "resin trap is a C-18 resin" from claim 103 is supported, for example, on page 30 line 20. The recitation of "kawain and methysticin" from claim 105 is supported, for example on page 32 line 11 and by Table 1. The other recitations of claim 105 and dependent claims thereof are supported, for example by the description on page 32, example 2 and on page 35. Increasing temperature 90 or more degrees as recited in claims 104, 109, 115 and 118 is supported, for example on page 36 lines 4-6. The high recovery of lactones recited in claim 110 is supported, for example on page 32 line 11 and Table 1. The recitation of an 80 degrees temperature in claim 113 is supported, for example on page 36 lines 4-6. The reduction of analysis time by a 7-10 % methanol in carbon dioxide gradient recited in claim 114 is supported, for example, on page 36 lines 10-15.

### The Prior Art Rejections

Castor U.S. No. 5,440,055 has been cited as exemplifying supercritical processes, and more specifically to the use of methanol to elute a taxol-containing mass from a column. The new claims recite more specific aspects of desired embodiments, which distinguish even more over the features of this cited art. For example, some conditions, that are specifically claimed in claims 105 through 115 were

found to isolate unexpectedly high amounts of kawain and methysticin, as described on page 32 (see line 11) and as seen in Table 1 of the specification.

Castor teaches the necessity of multiple solvents to obtain fractionation. See for example column 3 lines 26 to 47. Fractions are absorbed onto columns and then desorbed with new solvents. This process typically happens four times (column 3 lines 41 to 47). Fractions are then subjected to further purification (lines 48 to 62). In contrast, methods as claimed can both separate and isolate fractions of closely related bioactive substances in a single step after first extracting the material into a resin trap and eluting from the trap. The examiner also has cited multiple references that when combined, allegedly describe previous claims 17-19 and 79-93. The new claims, in contrast recite more specific details, many of which are not even found in the cited references.

Consideration of the new claims in view of their added specific details and other distinguishing features over the Castor and the cited art courteously is requested.

Respectfully submitted,

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99. A method for obtaining bioactive substances from plant material, comprising:  
mechanically disrupting the material by grinding, crushing, or macerating;  
contacting the disrupted material with supercritical fluid at high pressure to  
remove bioactive substances from the plant material;  
collecting the removed bioactive substances with a resin trap;  
eluting the bioactive substances from the resin trap;  
and separating the substances by supercritical fluid chromatography, wherein the  
supercritical fluid that contacts the disrupted material comprises carbon dioxide, alcohol  
and isopropyl amine as a secondary modifier.
100. The method of claim 99, wherein the plant material is Kava root.
101. The method of claim 99, wherein the plant material is leaves and bark of  
Byrsonima crassifolia.
102. The method of claim 99, wherein the supercritical fluid chromatography is  
carried out by passing the supercritical fluid through an NH2 column.
103. The method of claim 99, wherein the resin trap is a C-18 resin.
104. The method of claim 99, wherein the column is maintained at a temperature of at  
least 90 degrees.
105. A method for obtaining a high recovery of kawain and methysticin from kava root,  
comprising:  
mechanically disrupting the kava root by grinding, crushing, or macerating to  
prepare an extract;

contacting the disrupted material with supercritical carbon dioxide and an alcohol at a pressure of at least 350 atmospheres and separating the substances by supercritical fluid chromatography;

collecting the removed kawain and methysticin with a resin trap;

eluting the kawain and methysticin substances from the resin trap.

106. The method of claim 105, wherein the alcohol is ethanol at 15%.

107. The method of claim 105, wherein the chromatography is carried out at 60 degrees centigrade.

108. The method of claim 105, wherein the supercritical pressure is maintained between 350 to 450 atmospheres.

109. The method of claim 105, wherein the column is maintained at a temperature of at least 90 degrees centigrade.

110. A method for obtaining a high recovery of kavalactones p.32 line 11, Table 1] from kava root, comprising:

mechanically disrupting the kava root to prepare an extract by grinding, crushing, or macerating;

contacting the disrupted material with supercritical carbon dioxide and an alcohol at a pressure of at least 275 atmospheres and separating the substances by supercritical fluid chromatography over an NH<sub>2</sub> column;

collecting the removed kavalactones with a resin trap; and

eluting the kavalactones from the resin trap.

111. The method of claim 110, wherein 15% of the carbon dioxide by volume is replaced with ethanol.

112. The method of claim 110, wherein the extracted methysticin is further purified by supercritical chromatography over an NH<sub>2</sub> column.

113. The method of claim 110, wherein the NH<sub>2</sub> column is operated at a temperature above 40 degrees centigrade.

114. The method of claim 110, wherein the analysis time is reduced by using a methanol in carbon dioxide gradient of from 7% to 10%.

115. The method of claim 110, wherein the column is maintained at a temperature of at least 90 degrees centigrade.

116. A method for obtaining bioactive substances from a plant material, comprising:  
\_\_\_\_\_ mechanically disrupting the plant material by grinding, crushing, or macerating;  
\_\_\_\_\_ contacting the disrupted material with supercritical fluid of carbon dioxide with an alcohol at high pressure to remove the bioactive substances from the plant material;  
\_\_\_\_\_ collecting the removed bioactive substances with a resin trap;  
\_\_\_\_\_ eluting the bioactive substances from the resin trap and separating the substances by supercritical fluid chromatography over an NH<sub>2</sub> column,  
\_\_\_\_\_ wherein isopropyl amine is mixed into the alcohol prior to mixing the alcohol with carbon dioxide as a secondary modifier.

117. The method of claim 116, wherein the supercritical fluid in contact with the NH<sub>2</sub> column is carbon dioxide and alcohol.

118. The method of claim 116, wherein the column is maintained at a temperature of at least 90 degrees.

119. The method of claim 116, wherein the plant material is Kava root.

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